Progesterone ELISA Kit

Cat#: orb1173300 (User Manual)

Progesterone ELISA Kit

Size: 48 T/96 T
Lot #: Refer to product label
Detection range: 0.5 ng/mL-30 ng/mL
Sensitivity: 0.2 ng/mL
Precision: Intra-assay Precision: The CV (%)＜15%. Inter-assay Precision :The CV (%)＜15%
Recovery: The recovery ranged from 85% to 115% with an overall mean recovery of 100%.
Specificity: Progesterone ELISA Kit has high sensitivity and excellent specificity for detection of Progesterone. No significant cross-reactivity or interference between Progesterone and analogues was observed.
Applicable samples: Serum, Plasma
Storage: The unopened kit should be stored at 4℃ for 12 months, protected from light.

Assay Principle

Progesterone is an endogenous steroid and progestogen sex hormone involved in the menstrual cycle, pregnancy, and embryogenesis of humans and other species. It belongs to a group of steroid hormones called the progestogens, and is the major progestogen in the body. Progesterone is also a crucial metabolic intermediate in the production of other endogenous steroids, including the sex hormones and the corticosteroids, and plays an important role in brain function as a neurosteroid. Progesterone ELISA Kit employs the competitive inhibition enzyme immunoassay technique. The microtiter plate provided in this kit has been pre-coated with goat-anti-rabbit antibody. Standards or samples are added to the appropriate microtiter plate wells with an antibody specific for progesterone and Horseradish Peroxidase (HRP) conjugated progesterone. The competitive inhibition reaction is launched between with HRP labeled progesterone and unlabeled progesterone with the antibody. A substrate solution is added to the wells and the color develops in opposite to the amount of progesterone in the sample. The color development is stopped and the intensity of the color is measured.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Progesterone Standard	0.25 mL×6	0.5 mL×6	4℃
HRP Conjugated Progesterone	3 mL	6 mL	4℃
Progesterone Detect Antibody	3 mL	6 mL	4℃
HRP Substrate A	3.5 mL	7 mL	4℃, protected from light
HRP Substrate B	3.5 mL	7 mL	4℃, protected from light
Stop Solution	3.5 mL	7 mL	4℃
Wash Buffer(20×)	7.5 mL	15 mL	4℃
Progesterone Microplate	48 wells	96 wells	4℃
Plate Covers	1	2	RT

Note: Std1: 0 ng/mL; Std2: 0.5 ng/mL; Std3: 2 ng/mL; Std4: 6 ng/mL; Std5: 15 ng/mL; Std6: 30 ng/mL.

Materials Required but Not Supplied

·Microplate reader capable of measuring absorbance at 450 nm
·Multi channel pipette or automated microplate washer
·Incubator, refrigerated centrifuge
·Precision pipettes, disposable pipette tips
·Deionized water

Reagent Preparation

Note: Bring all reagents equilibrate to room temperature before use. If crystals have formed in the Buffer Concentrates, warm them gently until they completely dissolved.
1×Wash Buffer: Wash Buffer(20×) dilute with deionized water 1:20 to obtain the 1×Wash Buffer. Store at 4℃.

Sample Preparation

1. Serum: Use a serum separator tube and allow samples to clot for 30 min at room temperature before centrifugation for 15 min at 1,000 g. Remove serum and assay immediately or aliquot and store samples at -20℃. Avoid repeated freeze-thaw cycles.

2. Plasma: Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 min at 1,000 g within 30 min of collection. Assay immediately or aliquot and store samples at -20℃. Avoid repeated freeze-thaw cycles.

Note: Do not use grossly hemolyzed or lipemic specimens. If samples are to be used within 24 hours, they may be stored at 2 to 8℃. Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

Assay Procedure

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
2. Add 50 µL of Progesterone Standard or Sample per well. It is recommended that all Standards and Samples be added in duplicate to the microplate. Set a Blank well without any solution.
3.Add 50 µL of HRP Conjugated Progesterone to each well (not to Blank well), then add 50 µL of Progesterone Detect Antibody to each well in the same order. Mix well, cover with the plate cover provided and then incubate for 1 h at 37℃.
4. Remove liquid in each well and wash, repeating the process for a total of three washes. Wash by filling each well with 1×Wash Buffer (250 µL) using a Multi-channel pipette or automated microplate washer, and let it stand for 10 s, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining 1×Wash Buffer by invert the plate and blot it against clean paper towels.
5. Add 50 µL of Substrate A and 50 µL of Substrate B to each well, mix well and cover with the plate cover provided. Incubate for 15 min at 37℃. Keeping the plate away from drafts and other temperature fluctuations in the dark.
6. Add 50 µL of Stop Solution to each well. Stop Solution should be added to the plate in the same order as HRP Substrate. The color in the wells should change from blue to yellow. If the color in the wells is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
7. Determine the optical density of each well within 30 min, using a microplate reader set to 450 nm.

Data Analysis

1. Average the duplicate readings for each standard and sample.
2. Drawing of standard curve: With the standard solution concentration as the x-axis and the mean absorbance for each standard as the y-axis, draw the standard curve. A computer software can be used to create a standard curve.

Typical Data

Typical standard curve（R2≥0.99）

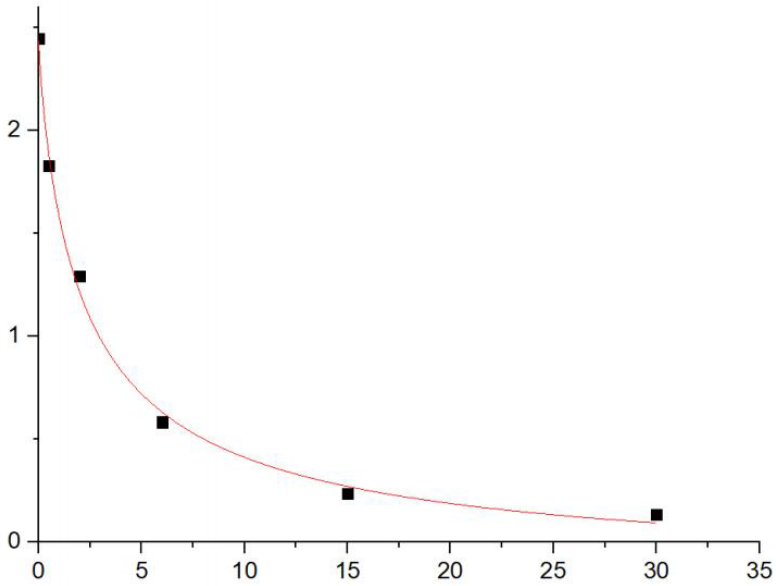

Figure1. Standard Curve of Progesterone in 96-well plate assay, data provided for demonstration purposes only. A new standard Curve must be generated for each assay.

Precautions

1. Do not mix or substitute reagents with those from other lots or sources.
2. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
3. To ensure accurate results, proper adhesion of plate covers during incubation steps is necessary.
4. Stop Solution has certain Corrosive. Please take protective measures when operating.